Chapter 11   Effects of nitrogen containing air pollutants: critical levels

The first edition of these guidelines *(1)* drew attention to the effects of nitrogen on vegetation. The guidelines for nitrogen-containing air pollutants were formulated as follows:

> In the presence of levels of sulfur dioxide and ozone not higher than 30 μg/m^3 and 60 μg/m^3 respectively, the atmospheric concentration of nitrogen dioxide should be no higher than 30 μg/m^3 as a yearly average of 24-hour means and no higher than 95 μg/m^3 as 4-hour average.

> In order to protect sensitive ecosystems, the total nitrogen deposition should not exceed 3 g/m^2 per year.

In this chapter new literature is reviewed in order to judge whether this guideline needs revision.

The atmosphere of the earth is 80% dinitrogen (N$_2$), which equals about 75×10^6 kg above each hectare of the earth's surface. In unpolluted conditions a small fraction (1–15 kg N/ha per year) is converted by nitrogen-fixing microorganisms to biologically more active forms of nitrogen: ammonium (NH$_4^+$) and nitrate (NO$_3^-$). The natural deposition of nitrogen-containing atmospheric compounds other than N$_2$ is much less. The earth itself contains five times more nitrogen than the atmosphere, but weathering of solid rock is a negligible source of biologically active nitrogen. By denitrification (reduction of NO$_3^-$ to N$_2$ and to a lesser extent nitrous oxide (N$_2$O) and ammonia (NH$_3$)), 1–30 kg N/ha per year is recycled from the earth to the atmosphere.

Human activities, both industrial and agricultural, have strongly increased the amount of biologically active nitrogen compounds, thereby disturbing the natural nitrogen cycle. Various forms of nitrogen pollute the air, mainly nitric oxide (NO), NO$_2$ and NH$_3$ as dry deposition, and NO$_3^-$ and NH$_4^+$ as wet deposition. Another contribution is from occult deposition (fog and clouds). There are many more nitrogen-containing air pollutants, but these are not covered in this chapter, either because their contribution to the total nitrogen deposition is supposed to be small or because their concentrations are probably far below effect thresholds.

Nitrogen-containing air pollutants can affect vegetation indirectly, via chemical reactions in the atmosphere, or directly after being deposited on vegetation, soil or water. The indirect pathway is largely ignored in this chapter, although it includes very relevant processes and should be taken into account when evaluating the entire impact of nitrogen-containing air pollutants: NO and NO$_2$ are precursors for tropospheric ozone (O$_3$), which acts both as a phytotoxin and a greenhouse gas. Dinitrogen oxide (N$_2$O) is a greenhouse gas and also contributes to the depletion of stratospheric O$_3$, resulting in increasing ultraviolet radiation. The direct impact of airborne nitrogen is due to toxic effects, eutrophication and acidification. Thresholds for eutrophication and soil acidification are discussed in Chapter 14.

Two different types of effect threshold exist: critical levels and critical loads. The *critical level* (CLE) is the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge. The *critical load* (CLO) is a quantitative estimate of an exposure (deposition) to one or more

pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge**.**

Generally, for nitrogen-containing air pollutants, CLEs are expressed in terms of exposure ($\mu g/m^3$ and exposure duration), while CLOs are expressed in terms of deposition (kg N/ha per year). Both the CLE and the CLO are intended to protect vegetation, and can be "translated" into each other if the deposition velocity is known. From this point of view it would be superfluous to assess both CLEs and CLOs. However, with the currently accepted approach, CLEs and CLOs are more or less complementary: CLEs focus on effect *thresholds* for short-term exposures (1 year or less), while CLOs focus on *safe* deposition quantities for the long term (1–100 years). CLEs are not intended to completely protect plants against adverse effects: No-observable-effect levels (NOELs) are usually lower. For instance, a CLE can be set at 5% yield reduction. Thus, simply owing to differences in definition, the CLE is generally higher than the CLO (Fig. 1).

Fig. 1. Hypothetical exposure/response relationships for nitrogen-containing air pollutants.
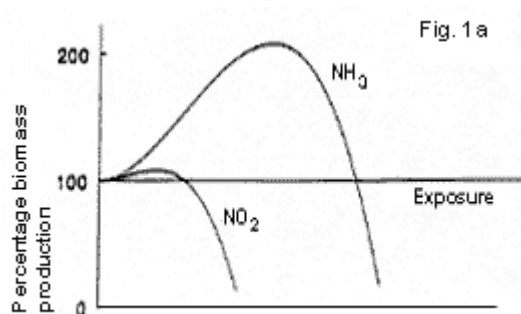

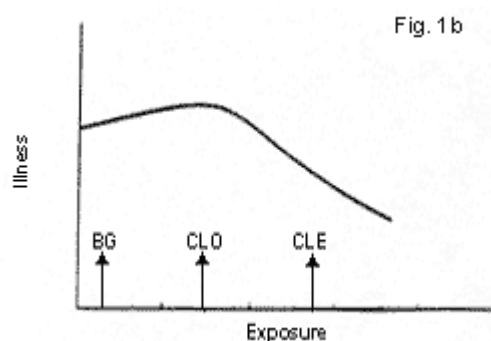**Fig. 1a. Biomass production related to exposure to NH$_y$ or NO$_x$.**

Fig. 1b. Fitness of a vegetation (e.g. expressed in vitality or species diversity) related to exposure.

BG: natural background; CLO: critical load; CLE: critical level.

In current practice there are other differences between CLEs and CLOs: CLEs give details on individual compounds and focus on responses at the plant level, while CLOs combine all nitrogen-containing compounds and focus on the vegetation or ecosystem level. In other words, CLOs focus on the functioning of the ecosystem, while CLEs focus on protecting relatively sensitive plant species.

In the CLE concept, the different nitrogen-containing compounds are evaluated separately because of their differences in phytotoxic properties, even when their load in terms of kg N/ha per year is the same *(2)*. Another difference between CLEs and CLOs is that CLEs consider the possibility of more- or less-than-additive effects *(3)*, while in the CLO concept additivity of nitrogen-containing or acidifying compounds is presumed. Moreover, nitrogen-containing air pollutants have their impact not only because of their contribution to the nitrogen supply. Sometimes other effects seem to dominatje. For instance, although occult deposition is generally small in terms of nitrogen deposition, it may be of great significance because of its ability to affect plant surfaces.

It was concluded for these reasons that both CLEs and CLOs are necessary in the scope of air quality guidelines for nitrogen-containing compounds.

Assessing effect thresholds is relatively simple in the case of toxic compounds with an exposure–response relationship that follows the usual sigmoidal curve: the lowest exposure level that results in a response significantly different from the control treatment is the effect threshold. Nevertheless, this approach is essentially invalid for exposure of nitrogen-limited vegetation to nitrogen-containing air pollutants. Nitrogen is a macro-nutrient, and thus each addition of nitrogen can result in a physiological response; growth stimulation gradually increases with higher exposure levels and changes in growth inhibition at higher levels (Fig. 1). Moreover, depending on the definition of "adverse effect", the status of the vegetation may not be at its optimum at background level (Fig 1). These features complicate the assessment of effect thresholds for nitrogen-containing compounds. Nevertheless, effect thresholds are presented in this chapter, as is current practice.

Properties of NO_x and NH_y [1]

Adsorption, uptake and re-emission

The impact of a pollutant on plants is determined by its adsorption and rate of uptake (flux) and the plants' reaction. Probably, foliar uptake is dominant for NO, NO_2 *(3)* and NH_3 *(4)*, while the pathway via soil and roots is the major route for nitrogen-containing pollutants in wet deposition. In this chapter, special attention will be paid to foliar uptake, while the pathway via the soil is evaluated in more detail in Chapter14.

The flux of compounds from the atmosphere into the plant is a complicated process, which is highly dependent on the properties of plant and compound and on environmental conditions. This is why deposition velocities proved to be highly variable (Table 1). In the case of NO, the level and variability of deposition velocity is also influenced by the fact that generally the sum of two opposite fluxes is measured: from the atmosphere into the vegetation and from the soil into the atmosphere. Uptake by the plant can occur, therefore, even when the effective deposition velocity is near zero.

Table 1. Deposition velocity of nitrogen-containing gases and aerosols

Compound	Deposition velocity (mm/s)	Sources
NO_2	1–8	*5,6*
NO	0–1	*7,8*
NH_3	12 (–5 to +30)	*9–11*
NH_4^+	1.4 (0.03 to 15)	*9*

According to Wellburn *(3)* and Grenfelt et al. *(6)*, NO_3^- and nitric acid (HNO_3) have a higher deposition velocity then NH_3, but this was not quantified. The flux from the atmosphere to the leaf surface (and soil) depends on the aerodynamic and boundary layer resistances, which are determined by meteorological conditions and plant and leaf architecture. The flux from the leaf surface to the final site of reaction in the cell is determined by stomatal, cuticular and

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[1] $NO_x = NO_2 + NO$; $NH_y = NH_3 + NH_4^+$.

mesophyll resistance. The reaction of the plant to the nitrogen that arrives at the target site depends on intrinsic plant properties and its nutritional status, and again on environmental conditions. The flux of atmospheric nitrogen through the soil is conditioned by properties of soil and vegetation and by meteorological conditions. The chemical composition of soil water, the nitrification rate ($NH_4^+ \rightarrow NO_3^-$), the preference of the plant for either NH_4^+ or NO_3^-, the root architecture and the metabolic activity of the plants play major roles in this uptake *(12)*.

Adsorption on the outer surface of the leaves certainly takes place. Exposure to relatively high gaseous NH_3 concentrations (180 µg/m^3) or NH_4^+ in rainwater (5 mmol/litre) damaged the crystalline structure of the epicuticular wax layer of the needles of *Pseudotsuga menziesii* *(13)*. NO_2 *(14)* and NH_4^+ and NO_3^- in wet and occult deposition can disturb leaf surfaces in several ways *(15)*. The quantitative relevance of this effect for field situations has not yet been proved in detail.

Uptake of NH_3 and NO_X is driven by the concentration gradient between atmosphere and mesophyll and mostly, but not always, is directly determined by stomatal conductance and thus depends on factors influencing stomatal aperture. Although in higher plants uptake through the stomata strongly dominates, there are indications that penetration through the cuticle is not completely negligible. This was demonstrated for NO and NO_2 *(3)*. While stomata strongly influence the foliar uptake of aerial nitrogen compounds, many of these subsequently alter stomatal aperture and the extent of further uptake. The nitrogen status of plants in general terms is also known to affect stomatal behaviour towards other environmental conditions such as drought *(16)*.

The flux of NH_3 into the plant appears to be linearly related to the atmospheric concentration *(17)*, with no mesophyll resistance. This relation can become less than linear with high concentrations (some hundreds of µg/m^3) *(18)*. Mesophyll resistance is, however, probably more significant for NO and NO_2 *(19)*.

There is increasing evidence that foliar uptake of nitrogen reduces the uptake of nitrogen by the roots *(4,20)*, although the driving mechanism is not yet clear.

In the presence of very low concentrations, plants can emit NH_3 rather than absorb it. This is especially true with scenescing and with highly fertilized plants *(9–11)*. Release to the atmosphere of N_2 and NO by plants has also been reported. In some cases this was part of the response on exposure to nitrogen-containing pollutants, but other mechanisms are involved as well *(3)*.

Rain, clouds, fog and aerosols always contain significant amounts of ions, including NH_4^+ and NO_3^-. In the past, foliar uptake of nitrogen from wet deposition was considered to be negligible, but recent research using ^{15}N and throughfall analysis shows that this path can contribute a high proportion of the total plant uptake *(21)*. In general, cations such as NH_4^+ are more easily taken up through the cuticle than are anions such as NO_3^-. A substantial foliar uptake of NH_4^+ from rainwater has been measured in several tree species *(22)*. Lower plants such as bryophytes and lichens do not have stomata and a waxy waterproof cuticle, and thus the potential for direct uptake of pollutants in the form of wet or dry deposition is greater. Epiphytic lichens are active absorbers of both NH_4^+ and NO_3^- *(23)*. Uptake and exchange of ions through the leaf surface is a relatively slow process, and thus is only relevant if the

surface remains wet for longer periods. Foliar uptake may, therefore, be important to vegetation that receives large amounts of wet and occult deposition, such as in mountainous regions.

Toxicity, detoxification and assimilation

One would expect a positive relationship between the solubility of a compound and its biological impact. NO is only slightly soluble in water, but the presence of other substances can alter it. NO_2 has a higher solubility, while that of NH_3 is much higher.

Much information exists on mechanisms of toxicity, although it is sometimes confusing. NO_2, NO, HNO_2 and HNO_3 can be incorporated in nitrogen metabolism using the pathway $NO_3^- \rightarrow NO_2^- \rightarrow (NH_3 \leftrightarrow NH_4^+) \leftrightarrow$ glutamate $\rightarrow$ glutamine $\rightarrow$ other amino acids, amides, proteins, polyamines, etc. The enzymes involved include nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS). Glutamate dehydrogenase (GDH) plays a role in internal cycling of NH_4^+.

After exposure to NO_2, nitrate can accumulate for some weeks; accumulation of nitrite is rarely reported, although it is surely an intermediate. Nitrite can be elevated for some hours owing to the fact that NR activity is induced faster than that of NiR. In many cases storage of excess nitrogen was found in the form of arginine *(24)*, which could last months or longer.

NO_2^-, NH_3 and NH_4^+ are all highly phytotoxic, and could well be the cause of adverse effects of nitrogen-containing air pollutants. Wellburn *(3)* suggested that the free radical •N=O plays a role in the phytotoxicity of NO_x.

More-than-additive effects (synergism) have been found in nearly all studies concerning SO_2 + NO_2 *(25)*. Inhibition of NiR by SO_2, resulting in the inability of the plant to detoxify nitrite, might be the cause of this interaction. High concentrations can cause visible injury via lipid breakdown and cellular plasmolysis. At lower concentrations inhibition of lipid biosynthesis may dominate, rather than damage of existing lipids *(3)*.

Raven *(26)* claimed that the adverse effects of nitrogen-containing compounds are due to their interference with the cellular acid/base regulation. They can influence cellular pH both before and after assimilation. Assimilation of most air pollutants, including NH_3, was proved to result in production of protons *(18)*. Assimilation of nitrate and a high buffer capacity can prevent the plant from being damaged by this acidification *(21)*. If these adverse effects can be effectively counteracted, assimilation of nitrogen-containing compounds may result in growth stimulation.

Physiology and growth aspects

When climatic conditions and nutrient supply allow biomass production, both NO_x and NH_y result in growth stimulation at low concentration and growth reduction at higher concentration. Nevertheless, the exposure level at which growth stimulation turns into growth inhibition is much lower for NO_x than for NH_y (Fig. 1).

Foliar uptake of NH_3 generally results in an increase in photosynthesis and dark respiration, and in the amounts of rubisco and chlorophyll. Some authors have shown that stomatal conductance increases in the dark, resulting in enhanced transpiration and drought sensitivity

(27). Most experiments with NO and NO_2 have been done with relatively high concentrations (> 200 µg/m^3). Those experiments show inhibition of photosynthesis by both NO and NO_2, possibly additively *(28)*. Inhibition by NO may be stronger than that by NO_2 *(29)*. The threshold for this response is well below the threshold for visible injury *(3)* and transpiration *(29)*. With lower, nearer to ambient NO_x concentrations, stimulation of photosynthesis may well occur. Both NO_x and NH_y generally cause an increase in the shoot–root ratio. The specific root length and the amount of mycorrhizal infection can be reduced by both compounds. These alterations in root properties resemble, however, general responses to increased nitrogen nutrient supply.

Interactions with climatic conditions
Evidence suggests that exposure of vegetation to NH_3 and to mixtures of NO_2 and SO_2 can influence the subsequent response to drought and frost stress. There is also evidence that environmental conditions can affect the response to NO_x and to NH_3.

The foliar uptake of nitrogenous compounds in the form of wet and occult deposition is largely via the cuticle. Uptake and exchange of ions through the leaf surface is a relatively slow process, and thus is especially relevant if the surface remains wet for longer periods, for instance in regions where exposure to mist and clouds is frequent.

The solubility of most gases, including NO, NO_2 and NH_3, is higher at lower temperatures, while the plant's metabolic activity (and thus its detoxification capacity) is lower. On the other hand, stomatal conductivity and thus the influx of gases is generally lower at lower temperatures.

Guderian *(30)* proposed a lower CLE in winter than for the whole year, in recognition of several results that indicate greater toxicity of NO_2 during winter conditions. For example, exposure of *Poa pratensis* in outdoor chambers to 120 µg/m^3 inhibited growth during winter but had little effect or stimulated growth in summer and autumn *(31)*. Mortensen *(32)* found that low light and non-injurious low temperatures can enhance the toxicity of NO_x. Caporn et al. *(33)* found that the depression relative to the control of net photosynthesis by 1250 µg/m^3 NO plus 575 µg/m^3 NO_2 at 10 °C was three times, and at 5 °C was almost five times that recorded at 20 °C. An interaction between NO_x and temperature may also occur at lower, more realistic concentrations. This is suggested by the observation of nitrite accumulation at low temperatures during fumigation of *Picea rubra* with 38 µg/m^3 NO_2 plus 54 µg/m^3 SO_2 *(34)*. This temperature effect may play a role in combination with elevated concentrations of CO_2 as well: the stimulating effect of CO_2 on net photosynthesis was inhibited by NO_x to a larger extent when applied at lower temperatures *(19)*. Observations of NH_3 injury to plants also indicate that this is greatest in winter *(35)*.

In contrast with the view that NO_x (and NH_3) injury is greatest at low temperatures, Srivastava et al. *(36)* found that inhibition by NO_x of photosynthesis was greatest under optimal temperature and high light conditions, when stomatal conductance to the gas would be highest.

The exposure of plants to NO_x and NH_3 may reduce their ability to withstand drought stress, owing to loss of control of transpiration by stomata and to an increase in the shoot–root ratio *(9)*.

Interactions with the habitat

Whether the atmospheric input of nitrogen has a positive or negative impact depends on the plant species and habitat. Based on experimental evidence, Pearson & Stewart *(21)* hypothesized that species that are part of a climax vegetation on nutrient-poor acidic soils are often relatively sensitive to NO_x and NH_y. Morgan et al. *(37)* found that NO_x disrupted the NR activity to a greater extent in calcifuge than in calcicole moss species. Ombrotrophic mires and other strongly nitrogen-limited systems may be especially prone to detrimental effects from input of nitrogen-containing air pollutants.

The assimilation of low concentrations of NO_2 by NR and incorporation into amino acids *(37)* are obvious indicators that this pollutant can contribute to the nitrogen budget of plants. Similarly, NH_3 can be assimilated by GS *(38)*. The contribution of NO_x to the nitrogen supply increases as root-available nitrogen is lowered *(39)*. Srivastava & Ormrod *(20)* observed reduced ability to respond to supply of nitrate to the roots when *Hordeum vulgare* was fumigated with NO_2. Similarly, with *Pinus sylvestris*, Pérez-Soba & van der Eerden *(4)* found reduced uptake of NH_4^+ from the soil when fumigated with NH_3. Although there is much evidence that nitrogen-containing air pollutants play a role in the nitrogen demand and nitrogen metabolism of the plant, Ashenden *(2)* found no obvious relationship between the sensitivity to NO_2 and the nitrogen preference as indicated by Ellenberg *(40)*.

Increasing pest incidence

Any change in the chemical composition of plants due to the uptake of nitrogenous air pollutants could alter the behaviour of pests and pathogens. Evidence indicates that, in general, NO_x and NH_y increase the growth rate of herbivorous insects *(17,41,42)*. This may apply to fungal pathogens as well *(43)*.

Nitrogen dioxide

Table 2 shows the lowest effective exposure levels for NO_2. Three different types of effect are considered:

- (bio)chemical: e.g. enzyme activity, chlorophyl content;

- physiological: e.g. CO_2 assimilation, stomatal conductivity; and

- growth aspects: e.g. biomass, reproduction, stress sensitivity.

Four exposure durations are used in Table 2. These are (including an indication of the exposure lengths and the margins):

- short-term (hours): < 8 hours

- air pollution episodes (days): 8 hours to 2 weeks

- growing season or winter season (months): 2 weeks to 6 months

- long-term (year(s)): > 6 months.

To avoid too selective information, in each cell a species is used only once. For each cell, the three lowest effective concentrations and exposure durations are given; species and references are mentioned in footnotes. Exposure levels far higher than current levels measured in the field situation have not been considered. In the case of discontinuous exposures, a concentration of 10 µg/m^3 is assumed during the hours that the fumigation was switched off.

This assumption may result in an overestimation of the mean if the experiments were performed in a very clean environment, or if the air filtration system was very effective.

Table 2: Lowest exposure concentrations (in µg/m^3) and durations at which NO$_2$ caused significant effects*

Exposure duration	Effect		
	(Bio)chemical	**Physiological**	**Growth aspects**
Long-term		128; 8 months[9]	85; 7 months[17]
			120; 5 months[18]
			122; 37 weeks[19]
Growing season or winter	50; 39 days[1]	120; 22 days[10]	10–43; 130 days[20]
	125; 140 days[2]	190 (65); 105 hours in 15 days[11]	55–75; 62 days[21]
	940; 19 days[3]		150–190 (28–33); 120 hours in 40 days[22]
Air pollution episodes	140; 1 day[4]	375 (165); 35 hours in 5 days[12]	375; 2 weeks[23]
	95; 7 days[5]	190; 20 hours[13]	100 (25); 20 hours in 5 days[24]
	65; 1 day[6]		
Short-term	7500; 6 hours[7]	190; 1 hour[14]	2000–3000; 3.5 hours[25]
	7500; 4 hours[8]	850; 7 hours[15]	
		1100; 1.5 hours[16]	

* If fumigation was not continuous, an average was estimated and is given in parentheses (calculated assuming a background concentration of 10 µg/m^3 during the periods of no fumigation).

[1] *Pinus sylvestris*: changes in amino acid composition, with no physiological changes *(44)*.
[2] *Lolium perenne*: increase in GDH activity *(25)*.
[3] *Lycopersicum esculentum*: decrease in nitrate content of the leaves *(45)*.
[4] *Picea rubens*: increase in NR activity *(46)*; in *Picea abies* Thoene et al. *(47)* found an increase of NiR after 1 day and a decrease after 3 days.
[5] *Azolla pinnata* in symbiosis with *Anabaena azollae*: increase in NR activity and decrease in spermine content *(48)*.
[6] Several bryophyte species: increase in NR activity *(37)*.
[7] *Zea mais*: increase in NiR activity *(49)*.
[8] *Vicia faba*: change in amino acid composition *(50)*.
[9] *Pseudotsuga mensiezii*: increased stomatal conductance and decreased water use efficiency *(13)*.
[10] *Betula* spp.: increased water loss *(51)*.
[11] *Phaseolus vulgaris*: reversible increase in dark respiration *(52)*; *Lycopersicum esculentum*: reduction of net photosynthesis *(28)*.
[12] *Glycine max*: increase in photosynthesis *(53)* and enhanced dark respiration *(54)*.
[13] *Phaseolus vulgaris*: increase in transpiration. In an experiment lasting 5 days, water loss per cm^2 leaf surface was measured every day. The proportional reduction was significant ($P < 0.01$) after 20 hours but decreased with longer exposure *(55)*.
[14] *Vicia faba*: increased growth rate of aphids following fumigation of the crop *(42)*.
[15] *Pisum sativum*: emission of stress ethylene *(56)*.
[16] *Medicago sativa, Avena sativa*: inhibition of photosynthesis *(57)*.
[17] *Lolium perenne*, *Phleum pratense, Dactylus glomerata*: in two partly similar experiments, general growth stimulation or no effect was found on shoot dry weight. In one experiment, *Lolium perenne* showed a 39% reduction after 3 months and 9% after 7 months *(58)*.
[18] *Betula pendula* was the only one of six tree species that showed a growth response (stimulation) *(31)*; *Citrus sinensis*: increased fruit drop at 120–500 µg/m^3 for 9.5 months *(59)*.
[19] *Polytrichum formosum* and three fern species: injury and changes in growth *(60,61)*.
[20] *Brassica napus* and *Hordeum vulgare*: growth stimulation *(62,63)*.

[21] *Phaseolus vulgaris*: increase in total dry matter, not in yield *(64)*.
[22] *Raphanus sativus*: growth stimulation *(65)*. With a similar exposure level (190 µg/m^3 for 4 hours per day for 35 days, resulting in a mean of 40 µg/m^3 at a background level of 10 µg/m^3) Yang et al. *(66)* found leaf damage in one out of three clones of *Pinus strobus.* Murray et al. *(67)* used 324 µg/m^3 for 4 hours per day for 108 days (mean 62 µg/m^3), which caused 50% inhibition of shoot growth in two *Medicago* species.
[23] *Helianthus annuus*: reduction in net assimilation rate (39).
[24] *Pinus strobus*: slight needle necrosis in two out of eight clones *(66)*.
[25] *Nicotiana tabacum*: leaf necrosis (68).

That not all cells in Table 2 are filled with three effective exposure levels is due to the fact that many of the experiments were done with unrealistically high concentrations.

The majority of observations, as mentioned in Table 2, are on crops; several of these show growth stimulation. Most of the responses on a biochemical level deal with enhanced NR activity, which shows that the plant is capable of assimilating the NO$_2$. A general effect threshold, as derived from Table 2, would be substantially higher if enhanced NR and biomass production of crops is not assumed to be an adverse effect. Nevertheless, growth stimulation is often considered an adverse effect in most types of natural vegetation. Moreover, Pearson & Stewart *(21)* assume detoxification of NH$_y$ and NO$_x$ as a potentially adverse effect, because it contributes to cellular acidification, which cannot always be counteracted.

Fig. 2 shows the data presented in Table 2. Two curves are drawn in the graph just below the lowest effective exposures. CLEs can be derived from this curve. The broken line indicates an absolute CLE according to current knowledge; no published information exists about effective exposures below this CLE. This approach is common in assessing CLEs for air pollution, although alternatives are being developed.

Fig. 2. Graphical presentation of the data given in Table 2: the lowest exposure levels of NO$_2$ affecting biochemical processes, physiology or growth. Curves are drawn below the lowest effective exposure levels. Black squares show the first edition of the WHO air quality guidelines *(1)*. X and Y axes are in log scale.
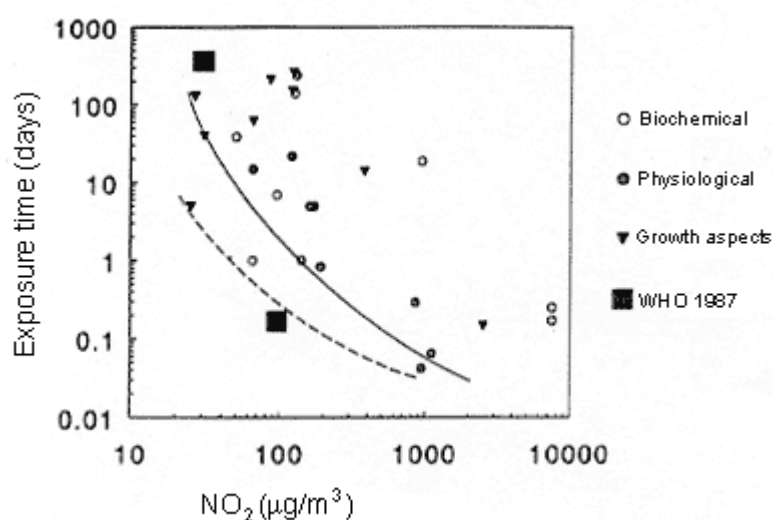

Fig. 2 shows that the standard for a 4-hour mean, as proposed in 1987 *(1)* is relatively stringent, and that its experimental basis is rather poor. The position of the broken line in Fig. 2 is strongly determined by one observation: slight needle necrosis in two out of eight clones

of pine seedlings *(66)*. Ignoring this observation, the CLE shifts to higher exposure levels. An indication for a CLE that is based on more observations is given by the solid line. This line suggests no-effect levels for 1 year, 1 day and 1 hour of around 20, 200 and 1000 $\mu g/m^3$, respectively. It is obvious that the estimation of CLEs using a selection of available information has arbitrary aspects.

Nitric oxide

Most research into the effects of NO has been done with glasshouse crops, particularly the tomato (*Lycopersicon esculentum*). Virtually all experiments deal with photosynthesis or enzymatic reactions, and a few growth aspects were measured. The existing data are also a little difficult to interpret, since controlled fumigation with NO inevitably results in some oxidation to NO_2, so atmospheres will contain a mixture of the oxides. There is growing interest in the distinct properties and effects of NO and NO_2, and the mechanisms of their cellular action probably differ *(3)*. The exchange properties of NO and NO_2 over vegetation (D. Fowler, personal communication) and single plants *(29)* appear quite different. Their effects are also contrasting, and there is clearly some dispute over which oxide is the more toxic. Earlier studies of the inhibition of photosynthesis found NO to act more rapidly than NO_2 (at several ppm) but to cause less overall depression of the photosynthetic rate *(57)*. In more recent photosynthesis studies with similar concentrations, Saxe *(69)* found NO to be considerably more toxic than NO_2. There is very little information on contrasting effects of the two oxides at low concentrations, but this also adds to the contention that NO is biologically more toxic. In their studies of NR in bryophytes, Morgan et al. *(37)* discovered that exposure to NO initially inhibited NR while NO_2 induced activity.

In long-term fumigation experiments, Lane & Bell *(58)* exposed several grass species to relatively low levels of NO, NO_2, SO_2, $SO_2 + NO_2$ and $NO + NO_2 + SO_2$. The NO concentration was 15 $\mu g/m^3$, and this generally caused no effect or growth stimulation, but in some cases growth reductions of more than 30% (included as footnote 15 in Table 3). An interactive effect of NO and $SO_2 + NO_2$ was observed in one case.

Table 3. Lowest exposure concentrations (in µg/m³) and durations at which NO caused significant effects

Exposure duration	Effect		
	(Bio)chemical	**Physiological**	**Growth aspects**
Long-term			
Growing season	44; 21 days[1]		625; 16 days[13]
	500; 28 days[2]		500; 35 days[14]
			15; 2.5–3 months[15]
Air pollution episodes	375; 8 days[3]	1250; 4 days[8]	1250; 5 days[16]
	44; 24 hours[4]	125; 20 hours[9]	
	1875; 18 hours[5]		

Short-term	188; 7 hours[6]	750; 1 hour[10]
	500; 3 hours[7]	2500; 10 min[11]
		125; 2 hours[12]

[1] Four bryophyte species: inhibition of nitrate-induction of NR *(37)*.
[2] *Lycopersicon esculentum*: induction of NiR *(25)*.
[3] *Lactuca sativa*: induction of NiR *(70)*.
[4] *Ctenidium molluscum* (bryophyte): inhibition of NR *(37)*.
[5] *Capsicum annuum*: reduction in NiR activity.
[6] *Pisum sativum*: increase in ethylene release *(56)*.
[7] *Lycopersicon esculentum*: induction of NiR *(25)*.
[8] Eight indoor ornamental species: inhibition of photosynthesis *(29)*.
[9] *Lycopersicon esculentum*: inhibition of photosynthesis *(28)*.
[10] *Avena sativa*, *Medicago sativa*: inhibition of photosynthesis *(57)*.
[11] *Lactuca sativa*: inhibition of photosynthesis *(71)*.
[12] *Lycopersicon esculentum*: inhibition of photosynthesis *(28)*.
[13] *Lactuca sativa*: reduction in plant mass *(33)*.
[14] *Lolium perenne*, *Phleum pratense*: 32–39% reduction in shoot growth (reduction disappeared after 7 months) *(58)*.
[15] *Lycopersicon esculentum*: reduction in plant mass *(72)*.
[16] *Lycopersicon esculentum*: reduction in plant mass *(73)*.

Fig. 3 shows that the experimental basis of the estimation of CLEs is poor. The CLE curve, which is based on four observations (and ignores one), suggests no-effect levels for 1 year, 1 day and 1 hour of around < 20, 100–200 and 200–1000 µg/m^3, respectively. A more relevant conclusion for these results may be that they need a better understanding of the effects of NO. At present, it is clear that NO cannot remain ignored as an air pollutant, as it can cause adverse effects at concentrations that frequently occur in many areas of Europe. Yet there is insufficient knowledge across a range of species to establish separate critical levels for NO and NO$_2$, and studies using a wider variety of vegetation are urgently required.

Fig. 3. Graphical presentation of the data given in Table 3: the lowest exposure levels of NO affecting biochemical processes, physiology or growth. Curves are drawn below the lowest effective exposure levels. X and Y axes are in log scale.
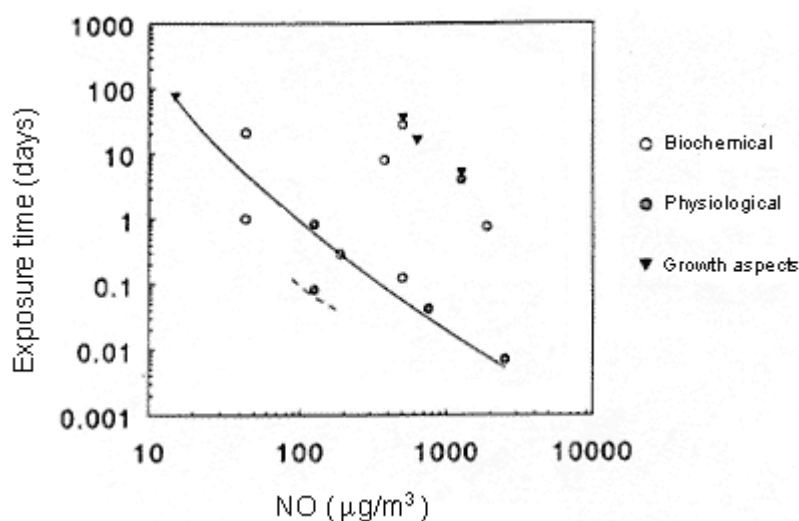
Ammonia

The toxicity of NH_3 at very short exposure times has been tested in the context of evaluating accidental releases during transport or industrial processes. An estimate of a CLE for 10 minutes is 150 μg/m^3 (J.A. Lee & A.W. Davison, personal communication). This type of exposure is outside the context of this chapter. Two cells in Table 4 could not be filled; the majority of quoted effects are on biomass production and tissue injury. From the references in Table 4, one should note that about 70% of the information originates from one Dutch research group. Only a few pollution climates were considered. The results may be representative for mild oceanic climates, but probably not for cold climates with dark winters; the toxicity of NH_3 increases with lower temperature and lower light intensity.

Table 4: lowest exposure concentrations (in μg/m^3) and durations at which NH$_3$ caused significant effects

Exposure duration	Effect		
	(Bio)chemical	Physiological	Growth aspects
Long-term	50; 8 months[1]	53; 9 months[7]	25; 1 year[10]
			53; 8 months[11]
			35; 16 months[12]
Growing season or winter	100; 6 weeks[2]	50; 6 weeks[8]	60; 2 months[13]
	60; 14 weeks[3]		20; 90 days[14]
	180; 13 weeks[4]		30; 23 days[15]
Air pollution episodes	2000; 24 hours[5]	213; 5 days[9]	120; 11 days[16]
	213; 5 days [6]		1000; 2 weeks[17]
			300; 3 days[18]
Short-term			30 000; 1 hour[19]
			2000; 2 hours[20]
			2000; 6 hours[21]

[1] Species of *Violion caninea* alliance: imbalanced nutrient status *(74)*.
[2] *Deschampsia flexuosa*: change in amino acid composition *(75)*.
[3] *Pinus sylvestris*: increased GS activity in Pine *(76)*.
[4] *Pseudotsuga menziesii*: imbalanced nutrient status *(13)*.
[5] *Lycopersicum esculentum*: increase of NH_4^+ in the dark *(35)*.
[6] *Lolium perenne*: 30% of nitrogen in the plant is derived from foliar uptake *(18)*.
[7] *Pinus sylvestris*: increased loss of water after two weeks of desiccation *(77)*.
[8] *Populus* spp.: increase in stomatal conductance in leaves; increase in mesophyll conductance and maximum photosynthetic rate at a slightly higher exposure level *(78)*.
[9] *Lolium perenne*: significant impact acid/base regulation and nutrients status *(18)*.
[10] *Pseudotsuga menziesii*: erosion of wax layer *(79)*; the authors have some doubts about the causality of this effect (personal communication).
[11] *Calluna vulgaris*: reduction in survival rate after winter *(80)*.
[12] *Arnica Montana*: reduction of survival and of flowering after winter *(17)*.
[13] Several conifer species: field exposure during winter; median concentration; severe injury *(35)*.
[14] *Viola canina, Agrostis capillaries*: 50% growth stimulation of the shoot (not of the roots) *(17)*.
[15] *Racomitrium lanuginosum*: chlorosis *(17)*.
[16] *Hypnum jutlandicum*: chlorosis *(17)*.
[17] *Lepidium sativum*: reduction in dry weight *(81)*.
[18] Several horticultural crops: leaf injury *(35)*.
[19] Various deciduous trees: leaf injury *(82)*.

[20] *Brassica* spp., *Helianthus* spp.: leaf injury *(83)*.
[21] *Rosa* spp.: leaf injury *(84)*.

Fig. 4 shows that the experimental basis for the estimation of CLEs is poor. No-effect levels for 1 year and 1 day may be in the range of 15–25 and 200–500 $\mu g/m^3$, respectively. As stated before, the observations cannot be considered to be representative to many parts of Europe. The effects of NH_3 should be studied on other plant species and in different climatic conditions in order to arrive at CLEs with a sufficient potential for generalization.

Fig. 4. Graphical presentation of the data given in Table 4: the lowest exposure levels of NH_3 affecting biochemical processes, physiology or growth. Curves are drawn below the lowest effective exposure levels. X and Y axes are in log scale.
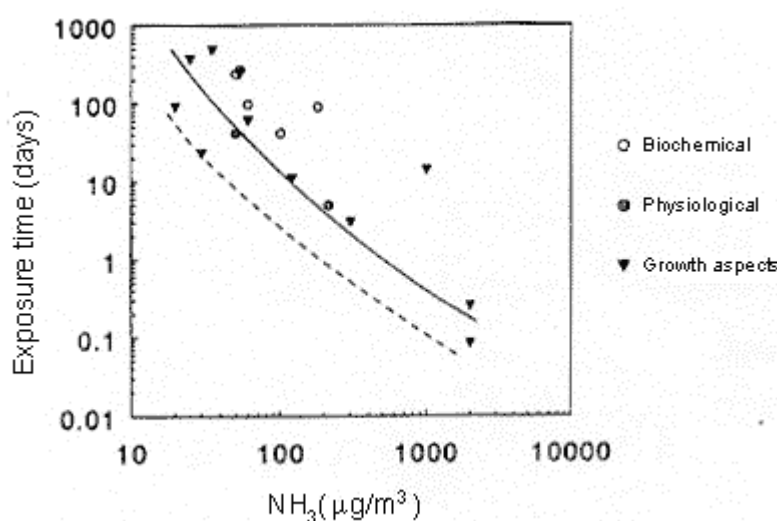
Ammonium and nitrate in wet and occult deposition

NH_4^+, NO_3^- and H^+ make up about half of the ionic composition of rain, clouds, fog and aerosols. The impact of the acidity of rain and clouds has received much attention in recent years *(15)*. This is not the case with other compounds in wet deposition, although their relevance is recognized. With the same pH, Cape et al. *(85)* found a much greater effect of sulfuric acid than of nitric acid, indicating that the impact of acid rain is not only through protons but also through anions.

There is an abundance of information on the effects of NH_4^+ in soil solution. However, threshold concentrations for NH_4^+ in the soil *(86)* cannot be supposed to be a CLE for rain water quality, because the type of exposure and response are completely different.

Wet deposition containing NH_4^+ can reduce frost tolerance *(87)* and induce leaching of K^+ and other cations *(88)*. It is not clear as yet whether this type of ion exchange can have deleterious effects by itself in the field situation.

Currently few quantitative data on the effects of nitrogen-containing wet and occult deposition are available for arriving at CLEs for this group of compounds.

Mixtures

A polluted atmosphere mostly consists of a cocktail of compounds, but some combinations are more probable. Owing to their role in the formation of tropospheric O_3, simultaneous co-occurrence of relatively high levels of O_3 and NO have been rarely observed, while sequential co-occurrences are much more frequent *(89)*. If burning of fossil fuels results in emission of SO_2, this is often combined with emission of NO_x.

Sulfur dioxide plus nitrogen dioxide
Synergism has been found in nearly all studies concerning this combination, with only few exceptions *(67,90,91)*. In general, it is to be expected that synergism disappears at higher exposure levels, owing simply to a collapse of plant function, including gas exchange.

Based on data presented by Whitmore *(92)* for *Poa pratensis*, the effect threshold for combinations of SO_2 and NO_2, in equal concentrations when expressed in ppm, is in the range of 1.2–2.0 ppm·days (Fig. 5). This threshold applies to effects of combinations of SO_2 and NO_2; the effects of single exposures were not assessed in this study. Nevertheless, it is reasonable from other references to expect synergism, and thus to include this threshold in Table 5, in which combination effects are summarized. The effect threshold for the effects of NO_2 + SO_2 on *Poa pratensis* is rather similar to that for *Hordeum vulgare*, which can be derived from the data of Pande & Mansfield *(93)*: 1.1–1.7 ppm·days. Another threshold for combinations of SO_2 and NO_2 is defined by van der Eerden & Duym *(94)* (Fig. 6). Information on combination effects derived from this source is included in Table 5.

Fig. 5. Effect of SO_2 + NO_2 on dry mass production of *Poa pratensis*, relative to control. Data are from several experiments in which the exposure periods and concentrations were in the range 20–100 days and 40–100 ppb, respectively
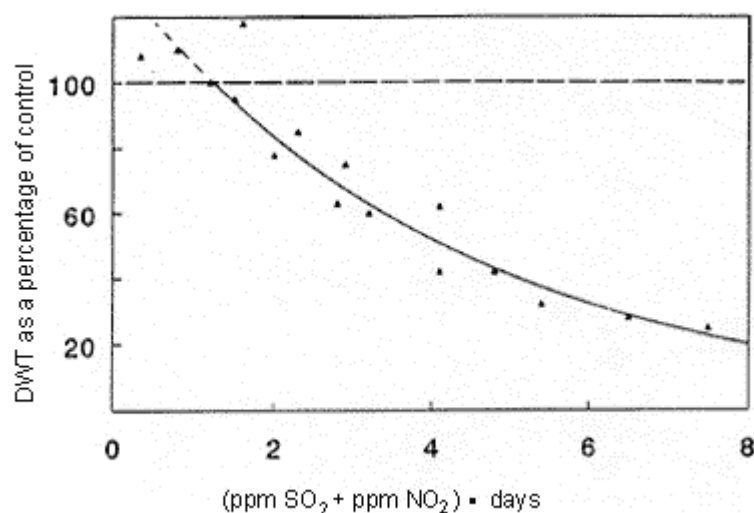
Source: Whitmore *(93)*.

Fig. 6. Threshold surface for combination effects of SO₂ and NO₂. Exposure levels above the surface are potentially toxic Derived from literature data of fumigations with different plant species
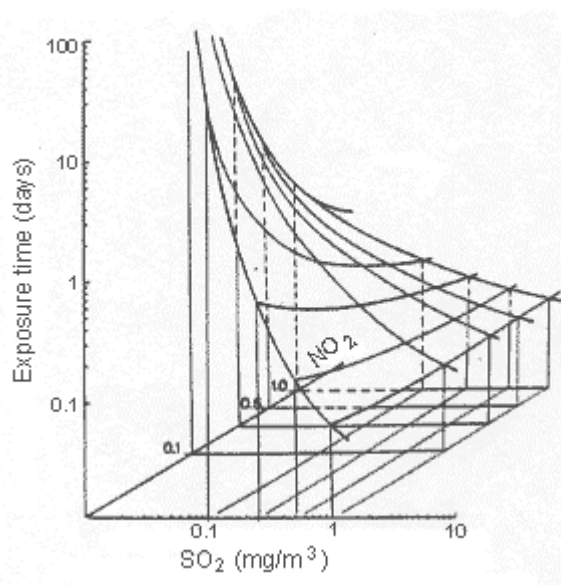
Source: van der Eerden & Duym (95).

Sulfur dioxide plus ammonia
Adsorption of either NH_3 or SO_2 on to the leaf surface is enhanced by the other *(78)*. Interactive physiological effects have also been found *(74,77,80)*. Currently, there is far too little information on this combination to quantify this interaction.

Nitric oxide plus nitrogen dioxide
When activated charcoal was used as filter material in NO_2 fumigation experiments, NO must have been present as well, because this material has little capacity to absorb NO. In those experiments responses must have been due to NO_2 + NO.

Although the toxicity of NO was often supposed to be much less than that of NO_2, currently the two compounds are assumed to be at least equally toxic and to act additively. Nevertheless, Wellburn *(3)* and others state that NO is more toxic, and Saxe *(69)* showed that the variation in sensitivity among species is different for the two compounds. This supports the suggestion of Wellburn that the mechanism of toxicity is different.

In the context of CLEs, the assumption of additivity of NO and NO_2 may result in an underestimation, but there are not enough data to quantify this.

Mixtures with ozone
The combination NH_3 + O_3 has rarely been studied. No statistically significant interactions have been found as yet, although in one study the threshold for leaf injury was higher in the presence of NH_3 *(95)*.

The combination $NO_2 + O_3$ has more frequently been studied. The responses differed strongly between experiments and species. Additivity or antagonism was found by Adaros et al. *(62,63)*, Bender et al. *(64)* and Runeckles & Palmer *(65)*. Synergism has been reported as well *(50,65,89)*.

The combination $SO_2 + O_3 + NO_2$ has also been studied. Again, the responses varied with plant species and experiment. Antagonism, additivity and synergism have all been found *(89,96)*. Steubing et al. *(97)* found in herbaceous species many relevant physiological effects (e.g. increased wettability of leaf surfaces of NO_2-treated plants (relative to control $SO_2 + NO_2$ and $SO_2 + NO_2 + O_3$ treatments) when fumigated with 100 µg/m^3 for only four hours per week during the growing season. There was no "NO_2 only" treatment in this experiment, and thus the results could not be included in Table 2 or Table 5, but it shows the ability of NO_2 to cause effects even at a small elevation of the mean concentration.

Table 5. Lowest exposure concentrations (in µg/m^3) and durations at which NO$_2$ increases the effect of SO$_2$, O$_3$, or SO$_2$ + O$_3$*

Exposure duration	Effect		
	(Bio)chemical	**Physiological**	**Growth aspects**
Long-term		128; 8 months[4]	150–190; 9 months[11]
			220; 60 weeks[12]
			19; 10–41 weeks[13]
Growing season or winter	55–75; 34 days[1]	135; 28 days[5]	30; 38 days[14]
	135; 28 days[2]		10–43; 130 days[15]
			30; 43 days[16]
Air pollution episodes	210; 1 days[3]	145 (24); 280 hours for 16 weeks[6]	80; 2 weeks[17]
		210; 2 days[7]	75; 1 day[18]
		190; 1–3 days[8]	210; 4 days[19]
Short-term		153; 1 hour[9]	325; 1 hour
		380; 1.8 hours[10]	400; 1 hour[20]

* See legend to Table 2.

[1] *Phaseolus vulgaris*: inhibition of parts of nitrogen metabolism when exposed in sequential exposure with O_3 (100–120 µg/m^3; 8 hours/day) *(64)*.

[2] *Lolium perenne*: decrease in proline content during winter hardening when applied in combination with SO_2 at 188 µg/m^3 *(98)*.

[3] *Solanum tuberosum*: changes in carbohydrate allocation when in combination with SO_2 at 300 µg/m^3 *(99)*.

[4] *Pseudotsuga menziesii*: reduced CO_2 assimilation when in combination with SO_2 at 129 µg/m^3 or NH_3 at 82 µg/m^3 *(13)*.

[5] *Lolium perenne*: less negative osmotic potential during winter hardening when applied in combination with SO_2 at 188 µg/m^3 *(98)*.

[6] *Araucaria cunninghamii*: several effects, including a decline of chlorophyll content; SO_2 at 250–450 µg/m^3 conteracted the effects *(90)*.

[7] *Solanum tuberosum*: effects on water potential of leaves and xylem when in combination with SO_2 at 300 µg/m^3 *(99)*.

[8] *Phaseolus vulgaris*: interaction with SO_2 (270 µg/m^3) in their impact on stomatal conductance *(55)*.

[9] *Phaseolus vulgaris*: Inhibition of photosynthesis when in combination with SO_2 at 215 µg/m^3; without SO_2 inhibition at 760 µg/m^3 *(100)*.

[10] *Glycine max*: inhibition of photosynthesis, synergism with SO_2 at 535 µg/m^3 *(101)*.

[11] Several crops: growth stimulation by NO_2 turns into a reduction in the case of combination with O_3 (160–200 µg/m^3; 6 hours/day; synergism of NO_2 + O_3) *(65)*.

[12] Six tree species: reduced plant growth in combination with SO_2 (280 µg/m^3), both antagonism and synergism *(102)*.

[13] Ten grass species were tested in combination with SO_2 (27 µg/m^3). Three species showed growth stimulation. Reduced growth was found at higher concentrations. Interactions with acidic mist and with O_3 were found *(2)*.

[14] *Poa pratensis*: inhibition of biomass production in combination with SO_2 (42 µg/m^3) for 38 days, the longest exposure period used in the experiments. Calculated from Whitmore *(92)*, assuming synergism and a CLE for SO_2 + NO_2 of 1.2 ppm·days.

[15] *Brassica napus, Hordeum vulgare*: antagonism (and rarely synergism) with O_3 (6–44 µg/m^3; 8 hours/day) and SO_2 (9–33 µg/m^3 continuously); enhanced yield turns into reduction *(62,63)*.

[16] *Plantago major*: reduced shoot dry weight synergism with SO_2 (60 µg/m^3) and O_3 (60 µg/m^3, 8 hours/day) *(103)*.

[17] *Poa pratensis*: inhibition of biomass production in combination with SO_2 (110 µg/m^3) for 2 weeks (the upper margin of the exposure period of this cell in the table; the shortest fumigation in this survey was 20 days). Calculated from Whitmore *(92)*, assuming synergism and a CLE for SO_2 + NO_2 of 1.2 ppm·days.

[18] CLE for NO_2 assuming SO_2 to be present at 70 µg/m^3; considered to be a CLE for a 24-h mean *(94)*.

[19] *Solanum tuberosum*: effects on root growth when in combination with SO_2 at 300 µg/m^3 *(99)*.

[20] *Lycopersicon esculentum*: reduction in plant mass if in combination with or preceded by O_3 (160 µg/m^3; 1 hour) *(104)*.

From Table 5 and Fig. 7 it seems somewhat surprising that the effective long-term exposures are generally higher than those of shorter duration. However, long term responses (more than half a year) have rarely been studied. Therefore, the information on effects over growing season periods may be much more representative for effects in the long term.

Fig. 7. Graphical presentation of the data given in Table 5: the lowest exposure levels at which NO_2 increases the effect of SO_2, O_3 or SO_2 + O_3. The curve is drawn below the lowest effective exposure levels. Black squares show the first edition of the WHO air quality guidelines *(1)*. X and Y axes are in log scale.
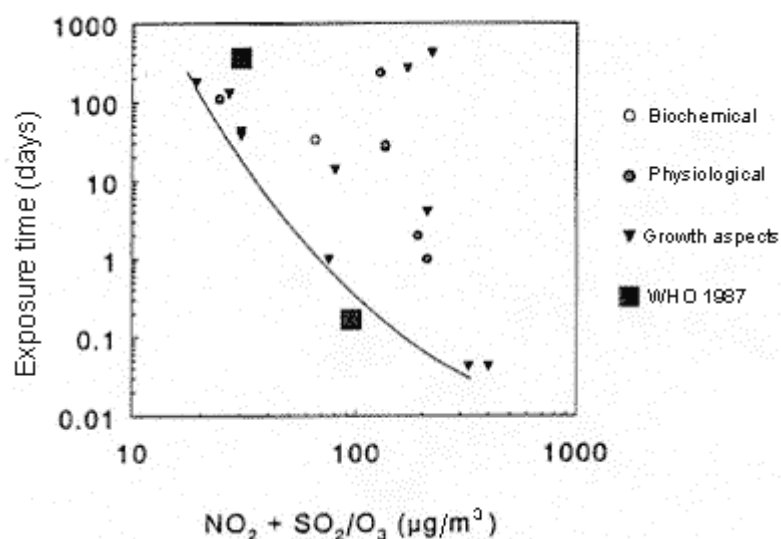
Ashmore (unpublished data, 1994) used 21 µg/m^3 and 11 µg/m^3 SO_2 and NO_2, respectively over the entire growing season and found synergism in reducing biomass production of *Pisum sativum* and *Spinacea oleracea*. Similar results were found for *Hordeum vulgare* and *Brassica oleracea* when fumigated for 120–190 days with 30–40 µg/m^3 SO_2 and 30–50 µg/m^3 NO_2. It is difficult to use these data for the assessment of CLEs, but they indicate that lower levels than those quoted in Table 5 can influence plant responses.

Mixtures with elevated carbon dioxide

Generally, an increased supply of CO_2 to crops results in enhanced biomass production. The responses of native species are more variable but frequently also positive. This growth stimulation is limited by deficiency of other nutrients. Nitrogen can be such a limiting factor, and for this reason a nitrogen fertilizer such as NH_y and possibly low NO_x concentrations could be hypothesized to have a more-than-additive relationship with CO_2. At present, however, there is no experimental evidence for this. van der Eerden et al. *(95)* and Pérez-Soba et al. *(38)* found that separate fumigations with NH_3 and CO_2 stimulated photosynthesis and growth, but not a combination.

Effects of combinations of NO_x and CO_2 have not yet been studied within the scope of global climate change, but some relevant information could be gained from the literature dealing with CO_2 enrichment in glasshouses. In using the flue gases of the heating system as a CO_2 source, NO_x (in which NO is dominant) becomes a major contaminant. The fertilizing effect of elevated CO_2 can largely disappear in the presence of a NO_x concentration of 1000 ppb or more *(19,32,72,73,105)*.

The CO_2, NH_3 and NO_x concentrations used in combination in these experiments are relatively high, and therefore cannot have any influence on the CLE assessment. Further experiments with lower concentrations are required.

Evaluation

Table 6 shows the former WHO guideline for NO_2 and some other CLEs assessed in the same period. Fig. 2, 3, 4 and 7 summarize the results given in Tables 2, 3, 4 and 5, respectively. In these figures, curves are drawn to estimate CLEs according to current practice, known as the "envelope" approach. Having plotted all effective exposure levels in a graph of concentration versus exposure time, a curve is drawn just below the lowest effective exposures, from which CLEs can be derived. The graphs indicate that more experiments with exposure periods of 0.5–5 days are required to give a more solid basis for estimating 24-hour CLEs.

A second approach to arriving at CLEs is the statistical model of Kooijman *(106)*. Based on the variation in sensitivity between species, CLEs are calculated taking into account the number of species tested and the level of uncertainty *(17)*. This approach is better, although the available data set is not suited to it.

Tables 2–5 show that some new relevant information has appeared. Comparing the data of Table 2 with those of Table 3 and Fig. 2 and 3, it appears that NO_2 has slightly higher effect thresholds than NO. However, this probably reflects the separate attention paid to these compounds rather then a difference in toxicity. It is now obvious that the toxicity of NO cannot be ignored, and it should be included in the guidelines. Currently, a guideline for NO_x (NO_2 + NO) is the most realistic approach to including the impact of NO, but future research should evaluate the specific phytotoxic properties of the individual compounds and their combinations.

It is not yet possible to discriminate in the CLE assessment between different types of vegetation such as crops, plantations, natural forests and other natural vegetation.
A 1-hour average for NO_2 of 800 $\mu g/m^3$ to prevent acute damage (Table 6) is probably too high; a CLE for NO_x of around 300 $\mu g/m^3$ would be better. A CLE of 95 $\mu g/m^3$ as a 4-hour

mean, as proposed in the former WHO guidelines *(1)* is still realistic, although rather stringent and not very practical. If CLEs for short periods (e.g. 1 or 8 hours) should be defined, it is probably necessary to separate daytime and night-time exposures. A CLE for a 24-hour mean is more practical, as this is related to both peak concentrations of some hours and to air pollution episodes of some days.

For the growing season and winter periods, Guderian *(30)* suggested a CLE of 60 and 40 $\mu g/m^3$, respectively. From Table 2 it can be seen that the CLE of 60 $\mu g/m^3$ can cause substantial growth stimulation rather than reduction. In the context of air quality guidelines, such a type of response must be regarded as potentially adverse, *inter alia* because of its influence on competition in natural vegetation. From current knowledge it is evident that 60 $\mu g/m^3$ is too high to prevent growth stimulation. Also, the CLE of 30 $\mu g/m^3$ for an annual mean *(1)* will almost certainly not protect all plant species, although for crops where growth stimulation is rarely an adverse effect this could be acceptable if secondary effects are negligible. The experimental basis for the previous WHO guidelines *(1)* was relatively poor, but evidence is increasing that they are not unrealistically low. Not even all direct adverse effects are eliminated by these levels *(2,62–64)*. Thus, the updated guidelines should be lower.

A long-term CLE for NO_2 of 10 $\mu g/m^3$, especially to avoid eutrophication of nutrient-poor vegetation, was proposed by Guderian *(30)* and Zierock et al. *(107)*. The basis for this proposal is the work of Lee et al. *(108)* and Press et al. *(109)*, who found growth reduction of *Sphagnum cuspidatum* after 140 days of exposure in regions with annual mean concentrations of 38 $\mu g/m^3$ and 11 $\mu g/m^3$, compared to the growth in another cleaner region (4 $\mu g/m^3$). Nevertheless, Lee et al. also showed that the poor growth of *Sphagnum* was more closely related to the excessively high concentrations of NH_4^+ and NO_3^- in bog water rather than to the concentration of NO_2 alone. So, this information could well be used to assess water quality standards, but is not directly useful as a basis for guidelines.

Table 6. Critical levels for NO_2 as cited in the literature

Concentration ($\mu g/m^3$)	Exposure time	Reference	Remarks
95	4 hours	WHO *(1)*	
30	annual mean	WHO *(1)*	SO_2 and O_3 not higher than 30 $\mu g/m^3$ and 60 $\mu g/m^3$, respectively
800	1 hour	Guderian *(30)*	
60	growing season	Guderian *(30)*	
40	winter	Guderian *(30)*	

Representativeness of the data
Critical levels for adverse effects of NH_3 on plants were estimated with the model of Kooijman *(17)*. To protect 95% of the species at $P < 0.05$, 24-hour and annual means of 270 $\mu g/m^3$ and 8 $\mu g/m^3$, respectively, were estimated. With the graphical approach, the 24-hour

average was a little lower and the annual mean somewhat higher (200–500 $\mu g/m^3$ and 15–25 $\mu g/m^3$, respectively (Fig. 4)).

Based on a review by Cape *(110)*, critical levels for H^+ and NH_4^+ were adopted for locations where ground-level cloud is present for more than 10% of the time and where Ca and Mg in rain or cloud do not exceed H^+ and NH_4^+ concentrations (mainly high elevation areas in cold climate zones): 300 $\mu mol/litre$ NH_4^+ as an annual mean *(111)*.

There remains a great deficiency in the amount and scope of experimentally derived information on which to base air quality guidelines. This is due to the fact that most experiments have been performed under conditions that cannot directly be compared with outdoor circumstances. In most experiments only primary effects such as photosynthesis and biomass production were evaluated, and rarely secondary effects such as alteration of stress tolerance or competitive ability. The plant species chosen in most experiments are often crops, although evidence suggests that some native species are relatively more sensitive. For instance, lower plants such as bryophytes and lichens are not protected by a waxy waterproof cuticle or the potential to close stomata. Furthermore, Pearson & Stewart *(21)* suggest that plant species from nutrient-poor acidic soils are more sensitive.

Further work, employing low concentrations of NH_y and NO_x (especially NO) in different climates, is urgently required. It is not realistic to screen for all likely growth and physico-chemical effects in the majority of species in order to arrive at general effect thresholds. Selections must be made on the basis of an understanding of differences in sensitivity between species. Nevertheless, an obvious mechanistic explanation for sensitivity differences is not yet available. For instance, there appears to be no relationship between sensitivity to NO_2 and nitrogen preference as indicated by Ellenberg *(2,40)*. Sensitivity classifications for some tens of species have been made for NO_2 and NH_3 *(112,113)*, but it appears difficult to extend predictions very far beyond those examined. The hypotheses of Raven *(26)* and Pearson & Stewart *(21)* should be studied in laboratory experiments and field studies in more detail, as this could result in an efficient selection criterion for further screening.

The assumption that all deposited nitrogen-containing compounds (which is part of the critical loads concept) act additionally in their impact on vegetation is poorly based on experimental results and is probably not valid for the short term. Generalizations and simplifications have to be made to arrive at conclusions that are applicable in environmental policy-making, but this should be done with great care. Mechanistic simulation models can become a powerful tool in making general predictions on the basis of various air pollution experiments *(114)*. Nevertheless, too much knowledge of biochemical and physiological mechanisms is still lacking to incorporate the impact of air pollution on vegetation into these models. This especially applies to natural vegetation, where stress sensitivity and competition are key factors.

Many gaps in understanding the impact of nitrogen-containing air pollutants on vegetation still exist, and this is a good reason to insert a safety factor in the CLEs and CLOs. However, there is currently still no broadly accepted approach to quantify such a safety factor.

General conclusions on critical levels

The file of information on gaseous NH_3 and on NH_4^+ in wet and occult deposition is still too limited to arrive at air quality guidelines, as they should have broad applicability. The CLEs for NH_3 and NH_4^+ are probably valid only for certain climatic zones.

In the majority of studies with NO and NO_2 there were no significant effects at levels below 100 $\mu g/m^3$ when applied singly, but in combination the effects are obvious. NO_2 changed the response to O_3 mainly with a less-than-additive interaction. In combination with SO_2, NO_2 acted more-than-additively in most cases. In general no interaction (and thus additivity) was found with CO_2 and with NO.

To include the impact of NO, we propose a CLE for NO_x instead of one for NO_2. To take combination effects with SO_2 and O_3 into account, the CLEs for these compounds are included in the CLE for NO_x.

In the first edition of these guidelines *(1)* a CLE for an annual average NO_2 concentration was 30 $\mu g/m^3$. Based on current information, we estimate the no-effect level for an annual average at around 15–20 $\mu g/m^3$ for NO_2, both when present as a single compound and in combination with SO_2 and O_3 (the nature of the NO_2 effect changes, but not the no-effect level). For NO a no-effect level for an annual average can only be estimated by extrapolation, but may well be around 15–20 $\mu g/m^3$ as well. Taking the additivity of NO and NO_2 effects into account, a CLE for NO_x that protects all plants from adverse effects should be lower than 15 $\mu g/m^3$. On the other hand, experimental evidence exists to indicate that the great majority of plant species (though not all) are protected at a NO_x level of 30 $\mu g/m^3$. We propose this level for the annual mean.

In the first edition a CLE for short-term exposure was $\mu g/m^3$ for a 4-hour mean. We propose to change this into a 24-hour mean. One reason for this change is that a 24-hour mean can be assumed to be related both to peak concentrations of some hours and to air pollution episodes of some days. Another reason is that both daytime and night-time exposures are included in the 24-hour mean. Experimental evidence exists that the CLE decreases from around 200 $\mu g/m^3$ to 75 $\mu g/m^3$ when in combination with O_3 or SO_2 at or above their critical levels. In the knowledge that short-term episodes of elevated NO_x concentrations are generally combined with elevated concentrations of O_3 or SO_2, 75 $\mu g/m^3$ is proposed for the 24 h mean.

This leads to a critical level for NO_x (NO + NO_2, expressed as NO_2 in $\mu g/m^3$) is 30 $\mu g/m^3$ as an annual mean and 75 $\mu g/m^3$ as a 24-hour mean.

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Fig. 1a

Fig. 1b